RESEARCH COMMUNICATIONS

REPAIR OF RADIATION-INDUCED STRAND BREAKS AS RELATED TO THE INDUCIBLE INHIBITOR OF POSTIRRADIATION DNA DEGRADATION

ASLIHAN TOLUN, RALPH CHRISTENSEN, *and* ERNEST C. POLLARD

From the Department of Biophysics, The Pennsylvania State University, University Park, Pennsylvania 16802

Recommended by W. Howard Cyr and Leo J. Grady

ABSTRACT The repair of radiation-produced single-strand breaks observed under alkaline conditions is very apparent in cells which possess an inducible inhibitor of postirradiation DNA degradation. Previous induction of the inhibitor with ultraviolet light increases the amount of repair. In those cells which are genetically not inducible there is no increase following ultraviolet irradiation.

Exposure of bacterial cells to ionizing radiation can be followed by the repair of lesions expressed as strand breaks under alkaline conditions (McGrath and Williams, 1966; Kapp and Smith, 1970) and degradation processes, in which the *rec*BC gene product (exonuclease V) plays an important role (Emmerson, 1968; Youngs and Bernstein, 1973). Postirradiation DNA degradation can be modified by the presence of an inducible inhibitor of DNA degradation (Grady and Pollard, 1968; Pollard and Randall, 1973). We report here experiments which suggest that the amount of repair of strand breaks is increased under conditions in which the inducible inhibitor is present.

The presence of an inducible inhibitor is manifested by the following signs: (*a*) a relatively rapid cessation of DNA degradation, complete by 30–40 min after irradiation; (*b*) extended degradation in the presence of rifampin and chloramphenicol; (*c*) extended degradation in starved amino acid auxotrophs (Pollard et al., 1974); (*d*) a dramatic decline in DNA degradation when the dose of ionizing radiation follows an inducing dose of ultraviolet (UV) radiation (about 300 ergs/mm^2 or less) given 20–60 min earlier (Pollard and Randall, 1973; Marsden et al., 1974). The results of applying these criteria to strains of *Escherichia coli* used in this study are shown in Table I.

TABLE I

CLASSIFICATION OF STRAINS USED ACCORDING TO CRITERIA GIVEN FOR DETECTION OF THE INDUCIBLE INHIBITOR

Strain	Relevant genotype	Source	Inducible inhibitor	Reference
B/r	Wild type	PSU* stocks	Yes	Pollard and Randall (1973)
B_{s-1}	*uvrA*$^-$ *exr*$^-$	PSU* stocks	No	Pollard and Randall (1973)
15T$^-$	Wild type	PSU* stocks	Yes	Grady and Pollard (1968)
15T$^-$ JG151	Lacks colicinogenic factor	PSU* stocks	No	Grady and Pollard (1968)
K12 P3478	*polA*$^-$	PSU* stocks	Yes	Pollard and Randall (1973)
K12 W3110	Wild type	PSU* stocks	Yes	Pollard and Randall (1973)
K12 AB2497T$^-$	Wild type	Dr. R. Boyce (U. Florida)	Yes	Pollard, Boyce, and Keller‡

*Pennsylvania State University.
‡In preparation.

Cells from a fresh stock were diluted 1:50 into 5 ml of Roberts' C-minimal medium (Roberts et al., 1955) supplemented with 2 g/liter casamino acids and grown at 37°C for 4–5 h. [^{3}H-methyl]thymidine (NET 027X, New England Nuclear Corp., Boston, Mass.) was used as a DNA label in all experiments, at 5 μCi/ml. It was added to the growth medium at the onset of incubation, except with P3478 where it was added 1.5 h before harvesting the cells. 250 μg/ml 2'-deoxyadenosine were added to B_{s-1} cultures to promote incorporation of the label. All other strains were auxotrophs for thymine and approximately 1.5 μg/ml cold thymine was added to these cultures for proper growth.

At an optical density (at 420 nm) of 0.3–0.35 (about 2×10^8 cells/ml) cells were centrifuged in a Sorvall ss-1 rotor (Ivan Sorvall, Inc., Newtown, Conn.) at room temperature and resuspended in medium containing only cold thymine (1–10 μg/ml). Some cultures were immediately recentrifuged and resuspended in fresh medium, excepting B_{s-1}, AB2497 and, in some cases, W3110 cultures which were allowed an extra 30 min incubation at this point. Cells were then iced, and after 10 min were irradiated with ^{69}Co gamma rays (Gammacell 200, Atomic Energy of Canada, Ltd., Ottawa, Ontario) while being bubbled with oxygen or nitrogen. Doses ranged from 8.2–42 krads. Controls were also iced.

Room temperature alkaline gradients (5–20% sucrose, 0.1 M NaCl, 0.01 M EDTA, pH 12.2) were layered gently with 0.1 ml of 0.1% sarkosyl + 0.01 M EDTA in 0.5 M NaOH and then with 0.05–0.10 ml cells (total cells about 5×10^6). Typically, one or two sets of three gradients were centrifuged simultaneously, one layered with a non-irradiated cell control, one layered with an irradiated, nonincubated sample, and one layered with an irradiated sample incubated for 60 min postirradiation prior to lysis. For the UV preirradiation of W3110, AB2497, and $B_{s-1}T^-$ two sets of three gradients were centrifuged simultaneously. All samples were allowed to lyse 15–30 min before centrifugation began. The time of centrifugation was 100 min at 20°C and 30,000 rpm in a SW 50L rotor. Gradients were fractionated into approximately 30 fractions, col-

lected directly into Triton X scintillant, and counted for 5–10 min/fraction in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.). This *total* activity, rather than acid-insoluble activity, was assayed, thereby allowing us a crude estimate of the amount of DNA degraded. Experiments in which both acid-insoluble and total activity were measured (Fig. 1) indicated that significant activity differences between the assays occurred only in the top few fractions, where acid-insoluble activity is expected.

The upper two panels of Fig. 1 show the findings on *E. coli* strain K12 W3110. These experiments were designed to give an idea of the difference between the observation of total activity and that of the acid-insoluble fraction. The left-hand panel shows results obtained by alternate sampling with TCA precipitation in the manner used by McGrath and Williams (1966). The right-hand panel shows the findings for the alternate samplings using the whole-cell technique. Only the few fractions at the top of the gradient show any difference.

Strain W3110 was found by Pollard and Randall (1973) to exhibit the inducible inhibitor. However, the strain also shows considerable inhibition of postirradiation DNA degradation without any preliminary induction. The results of Fig. 1 show that immediately after irradiation with 25 krads ^{60}Co gamma rays there is considerable fragmentation of the DNA as shown by the broad peak around fraction no. 18. However, upon incubation for 10 min there is a diminution in the size of the broad peak with a buildup at larger sizes and also at the top of the gradient. There is thus both a repair of DNA and further degradation as well. In order to permit tabulation of the results on several strains of cells the proportion of repaired DNA, of unchanged DNA, and degraded DNA have been estimated. The areas shaded as indicated on Fig. 1 (upper right panel) correspond to these three proportions.

In Fig. 1, middle panels, we show the comparison between two strains of *E. coli* 15T$^-$. The cells on the left-hand side of the figure have the inducible inhibitor; those on the right-hand side do not. The dose given is low; nevertheless, it can be seen in the left-hand panel that radioactivity indicative of DNA occurs at lower sizes after irradiation and that upon incubation the activity is found near the normal peak. Estimated values of molecular weights, using Studier's (1965) method, are indicated by the arrows. On the other hand, strain JG151, shown on the right, while showing fragmentation for the nonincubated case, shows no more than the considerable increase in the fractions at the top of the column and no significant return to the normal peak. The experiments have been repeated with nitrogenation and at different doses, with essentially similar results. We conclude that no repair can be seen in strain JG151 but it can be seen in 15T$^-$.

In the lowest panels in Fig. 1 we show the data taken on P3478 (*pol*A$_1^-$) in log phase (left) and after previous treatment with 230 ergs/mm^2 of UV light followed by incubation for 60 min (right). It can be seen that in the log phase cells irradiation produces a shift toward smaller sizes and no appreciable return to the normal peak, in agreement with Paterson et al. (1971). In the stationary phase case (data not shown) for a somewhat higher dose there is some evidence of a shift back to the normal.

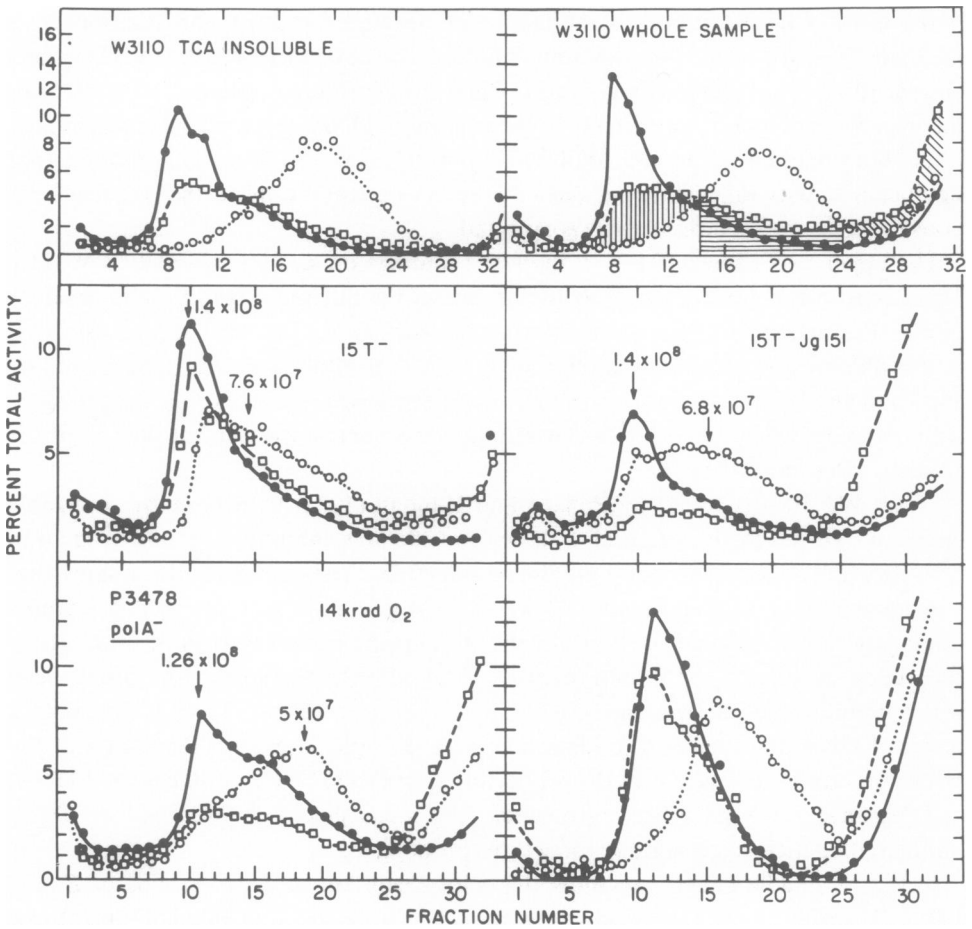

FIGURE 1 *Upper panels:* Gradient analysis of the DNA of cells of W3110 lysed after no radiation (●); after 25 krads oxygenated (○); and after 10 min postirradiation incubation (□). The left-hand panel shows alternate sampling in which the TCA-insoluble DNA is observed; the right-hand panel, those in which the whole cell radioactivity is counted. There is no appreciable difference except at the top of the gradient. In the right-hand panel the shading designates three regions used in tabulation of data: vertical, considered repaired; horizontal, unchanged; slanted, considered degraded. *Middle panels:* Gradient analysis of the DNA of strain $15T^-$ which has the inducible inhibitor (left), and strain $15T^-$ JG151 which does not (right). Unirradiated (●); irradiated with 8 krads and lysed without incubation (○); incubated 60 min (□). Approximate molecular weights shown are calculated by the Studier (1965) formula. Repair is observed in $15T^-$ but not in JG151. *Lower panels:* The log phase cells of P3478 (*pol* A^-) show little repair (left). Unirradiated (●); irradiated and lysed without incubation (○); incubated 60 min (□). P3478 cells (right) which received 230 ergs/mm^2 UV with 1 h incubation prior to irradiation. Unirradiated (●); irradiated and lysed without incubation (○); incubated 60 min (□). Repair is clearly apparent.

With UV light followed by 60 min incubation the DNA degradation is 92% inhibited (Pollard and Randall, 1973). It can be seen that there is a clear return from the small size to the normal distribution, good evidence for repair.

In order to show that the action of previous UV induction bears some relationship to the inducible inhibitor we additionally examined three strains of cells by the same procedure as used for P3478, exposure to UV followed by incubation for 60 min at 37°C and then irradiation with gamma rays. We used W3110 (*pol*A$^+$), the parent of P3478, AB2497, the thymine-requiring derivate of AB1157, the parent strain of many radiation-sensitive mutants, and B_{s-1} *thy*$^-$ *met*$^-$. The last is *exr*$^-$ and *uvr*$^-$ and does not show the inducible inhibitor (Pollard and Randall, 1973). AB2497 should show very definite inhibition after UV, W3110 should show only a moderate amount, and B_{s-1} none at all.

The findings are given in Table II, which also summarizes the experiments reported here. Some data for B/r are included for comparison. For AB2497 the preliminary UV dose increases the repaired fraction from 12–21% and diminishes the degraded fraction almost equally. For W3110 there is a slight increase in the repaired fraction, already quite high, and a diminution in the degraded fraction. For B_{s-1} there is no

TABLE II

SUMMARY OF GRADIENT EXPERIMENTS

Strain and conditions	Inhibitor status	Dose	Percent repaired	Percent unchanged	Percent degraded
		krads			
15T$^-$	+	8	15	40	3
15JG151	–	8	0	18	24
B/r, O_2	+	15	14	28	10
B_{s-1}, O_2	–	10	0	26	35
B_{s-1}, N_2	–	20	0	13	31
B_{s-1}, T$^-$	–	15	0	30	16
B_{s-1} + UV	–	15	3	13	31
AB2497	+	40	12	21	20
AB2497 + UV	++	40	21	23	14
W3110	+	25	24	25	15
W3110	+	36	26	25	30
W3110 + UV	+	36	27	21	10
P3478 log phase	±	14	2	31	17
P3478 stationary phase	+	22	4	18	17
P3478 + UV	++	22	19	11	12
P3478 + UV	++	22	28	26	9

The percent of activity counts in the repaired, unchanged, and degraded fractions was estimated as indicated in the upper right-hand panel of Fig. 1. Uncertainty in the estimates of 5% must be assumed. Note that the total percentage in the three areas does not include all the DNA fractions and so does not add up to 100%. Also the dose given differs for different strains because the sensitivity to DNA degradation also varies from strain to strain. A dose which gave approximately 50% degradation after 1 h was used.

diminution in the percent degraded; indeed there is degradation following UV alone and only a slight indication of any repair.

The exact nature of the inducible inhibitor is not known. It may be a gene product of *exr* or *lex* which inhibits exonuclease V (Marsden et al., 1974) or it may be a protein which binds to DNA and protects it from degradation. Whatever its nature, the experiments described here suggest that those strains in which induction of the inhibitor can occur, show more repair of single-strand breaks as examined by this technique. It is likely that DNA which has begun the process of degradation is not repairable and so the presence of inhibition of exonuclease V causes more DNA to be available for repair. Our experiments do not permit the conclusion that the inhibitor is itself a repair mechanism; on the other hand, the finding that there is more repair observed after induction of the inhibitor does not exclude the presence of abnormal amounts of repair of slowly repaired or nearly unrepairable lesions. These could be the mutation-producing repair events which are associated with inducible error-prone repair (Witkin, 1974; Witkin and George, 1973; George et al., 1974). More work is needed on this point.

We acknowledge help in discussion with B. K. Lydersen and technical help by E. Randall. Several gradients were run in the laboratory of Dr. P. Achey (University of Florida, Gainesville) who was most helpful in supplying equipment and expertise.

This work was supported by Atomic Energy Commission contract AT(11-1)-3411. R. Christensen was supported by National Institutes of Health Training Grant GM01015.

Received for publication 19 July 1974.

REFERENCES

EMMERSON, P. T. 1968. *Genetics.* **60**:19.

GEORGE, J., R. DEVORET, and M. RADMAN. 1974. *Proc. Natl. Acad. Sci. U.S.A.* **71**:144.

GRADY, L. J., and E. C. POLLARD. 1968. *Radiat. Res.* **36**:68.

KAPP, D. S., and K. C. SMITH. 1970. *J. Bacteriol.* **103**:49.

MCGRATH, R. A., and R. W. WILLIAMS. 1966. *Nature (Lond.)* **212**:534.

MARSDEN, H., E. C. POLLARD, W. GINOZA, and E. P. RANDALL. 1974. *J. Bacteriol.* **118**:465.

PATERSON, M. C., I. M. BOYLE, and R. B. SETLOW. 1971. *J. Bacteriol.* **107**:61.

POLLARD, E. C., D. BILLEN, and M. ACHEY. 1974. *Int. J. Radiat. Biol.* In press.

POLLARD, E. C., and E. P. RANDALL. 1973. *Radiat. Res.* **55**:265.

ROBERTS, R. B., D. B. COWIE, P. H. ABELSON, E. T. BOLTON, and R. J. BRITTEN. 1955. Carnegie Institution of Washington Publication 607.

STUDIER, F. W. 1965. *J. Mol. Biol.* **11**:373.

WITKIN, E. M. 1974. *Proc. Natl. Acad. Sci. U.S.A.* In press.

WITKIN, E. M., and D. L. GEORGE. 1973. *Genetics Suppl.* **73**:91.

YOUNGS, D. A., and I. A. BERNSTEIN. 1973. *J. Bacteriol.* **113**:901.